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Mechanisms of Drug-induced Delayed-type Hypersensitivity Reactions in the Skin

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ABSTRACT

Cutaneous drug reactions (CDRs) are the most commonly reported adverse drug reactions. These reactions can range from mildly discomforting to life threatening. CDRs can arise either from immunological or nonimmunological mechanisms, though the preponderance of evidence suggests an important role for immunological responses. Some cutaneous eruptions appear shortly after drug intake, while others are not manifested until 7 to 10 days after initiation of therapy and are consistent with delayed-type hypersensitivity. This review discusses critical steps in the initiation of delayed-type hypersensitivity reactions in the skin, which include protein haptenation, dendritic cell activation/migration and T-cell propagation. Recently, an alternative mechanism of drug presentation has been postulated that does not require bioactivation of the parent drug or antigen processing to elicit a drug-specific T-cell response. This review also discusses the role of various immune-mediators, such as cytokines, nitric oxide, and reactive oxygen species, in the development of delayed-type drug hypersensitivity reactions in skin. As keratinocytes have been shown to play a crucial role in the initiation and propagation of cutaneous immune responses, we also discuss the means by which these cells may initiate or modulate CDRs.

KEYWORDS: cutaneous drug reactions, delayed-type hypersensitivity, dendritic cells, keratinocytes, T-cells, cytokines

INTRODUCTION

Adverse drug reactions (ADRs) are very common in clinical practice.[1] ADRs are noxious or unintended reactions to a drug that is administered in standard doses by the proper route for the purpose of prophylaxis, diagnosis, or treatment of a specific disease.[2] For some drugs, ADRs occur in a large portion of those who receive the drug (eg, nausea associated with oncolytic agents). Other reactions occur in only a small portion of patients receiving the agent and it is not possible to identify in advance who will experience such reactions (eg, anaphylaxis after penicillin administration). Among various target organs, skin is one of the most frequent sites for ADRs.[3,4] Although numerous mechanisms have been suggested for cutaneous drug reactions (CDRs), experimental evidence for the mechanism of these reactions is limited.

Many of the cutaneous reactions associated with drug therapy are believed to be immune-mediated. Such reactions can be divided into 2 main categories: immediate-type or delayed-type immune-mediated reactions[4] . These CDRs are mediated either by immunoglobulin E (IgE; immediate-type reactions) or T cells (often referred to as delayed-type hypersensitivity [DTH]).[5] While the mechanism of IgE-mediated reactions is better understood, the genetic basis for predisposition to these reactions following drug administration (eg, penicillin) is unclear.[1] Less well understood is the mechanism by which DTH reactions occur in the skin after the administration of drugs systemically. In this review, we discuss critical steps and factors associated with DTH reactions in skin and discuss potential mechanisms by which these may be provoked after systemic drug administration.

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CHARACTERIZATION OF DELAYED-TYPE HYPERSENSITIVITY REACTIONS IN SKIN

Contact Dermatitis

Allergic contact dermatitis (ACD) is one of the most studied forms of xenobiotic-induced DTH, in which topical application and elicitation of sensitization to xenobiotics is confined to the skin. Eczema and dermatitis are used synonymously to denote the polymorphous pattern of skin inflammation characterized in its acute phase by erythema, vesiculation, and pruritus.[6] ACD is mostly caused by small molecules (<500 d), generally denoted as haptens. A variety of chemicals can act as haptens and cause ACD, including pharmaceutical,[7] industrial (eg, hair dyes or fragrances),[8,9] and metallic compounds (eg, nickel sulfate).[10] These small molecules can easily penetrate the stratum corneum of the skin barrier and interact with cutaneous proteins to form covalent adducts. Most haptens are weak allergens, requiring repeated exposures before they cause sensitization. However, strong

allergens such urushiol (the allergenic component of poison ivy) can produce sensitization after a single exposure.[11]

In addition to conventional ACD, there are also cases of systemic contact dermatitis, which is an inflammatory skin reaction occasionally seen as a flare up of previous dermatitis upon systemic exposure of allergen-sensitive individuals to the hapten (ie, administered orally, intravenously, or by inhalation).[12] In one study, it was demonstrated that some subjects (13 out of 33) with a history of nickel-induced contact dermatitis develop dermatitis when challenged with nickel orally, while no subjects without a skin sensitivity to nickel exhibited a cutaneous reaction.[12] These results suggest that cutaneous sensitization to a xenobiotic may result in a skin reaction upon subsequent systemic exposure to the same xenobiotic.

Delayed-type Hypersensitivity Reactions in the Skin After Systemic Drug Administration

DTH reactions in skin are also associated with the systemic administration of a wide number of drugs. Such drugs include antimicrobial (eg, sulfonamides, dapsone), anticonvulsant (eg, carbamazepine), anesthetic (eg, lidocaine), analgesic (eg, acetaminophen), antipsychotic (eg, clozapine), cardiovascular (eg, procainamide and hydralazine), nonsteroidal anti-inflammatory (eg, diclofenac), and steroid (eg, estrogen) agents. Naldi et al[13] found that antimicrobial drugs are the most common agents associated with CDRs, followed by nonsteroidal anti-inflammatory drugs (NSAIDs) and other analgesics. However, CDRs in response to NSAID and analgesic therapy were more commonly associated with fatalities than those occurring with antimicrobial agents. These investigators also found that the most frequently reported serious antimicrobial induced-CDRs were angioedema, erythema multiforme, and Stevens Johnson Syndrome.

DTH reactions in skin observed after systemic drug administration generally occur 7 to 10 days after initiation of therapy and are commonly associated with fever.[14] While rash is the most common manifestation observed with these reactions, systemic organ involvement (eg, liver and kidney) is also observed.[15] Most CDRs rapidly subside upon removal of the offending agent. In some instances, however, drug rashes progress despite discontinuance of the drug.

Accurate causality assessment is an important issue for clinicians and scientists using human subjects to investigate the mechanisms of CDRs.[16,17] As many patients experiencing an acute skin eruption are receiving several drugs, identification of the causative agent is problematic. The ideal "proof" that an agent caused the skin eruption in a given patient is reappearance of the reaction upon re-exposure to the suspected agent. However, in most patients therapeutic need does not justify the risk of re-exposure. Hence, definitive proof of the offending agent is most commonly lacking. As a consequence, diagnosis is based on probability assessment using the clinical history, timing of administration, and knowledge of previous associations of similar reactions with the suspected drug.[17]

Evidence Suggesting the Involvement of the Immune System in Cutaneous Drug Reactions

Numerous lines of evidence, mainly derived from immunohistochemical analysis of skin lesions and generation of drug specific T-cell clones from individuals with a history of a CDR, indicate that the cell-mediated component of the immune system plays a central role in the pathogenesis of drug-induced DTH reactions in the skin.[18-20] Moreover, the delayed onset, swelling of lymph nodes,[21] and secretion of pro-inflammatory cytokines[22,23] during these reactions provide further support for the direct involvement of the immune system in these CDRs. Suppression of drug-induced hypersensitivity reactions by gradual dose escalation, which may be explained by immune tolerance, also suggests a direct involvement of the immune system in the pathogenesis of drug-induced DTH.[3]

Drug-induced skin reactions appear most frequently as exanthemas (a widespread rash or eruption). Using skin-tests and immunohistochemical analysis, Britschgi et al[24] demonstrated a massive infiltration of neutrophils in pustules and T cells in the dermis and epidermis from patients with exanthematous pustulosis. Immunohistological analysis of skin at the time of bromisovalum-induced CDR also showed infiltration of CD4+ and CD8+ lymphocytes in dermal and epidermal infiltrates, respectively.[25] Histological analysis of other drug-induced exanthemas have also showed mild to moderate mononuclear cell infiltration in the skin.[18] These mononuclear cells are composed of CD3+ T cells with a predominance of CD4+ T cells in the perivascular dermis, whereas an equal number of CD4+ and CD8+ T cells appear in the dermato-epidermal junction zone and epidermis of skin.[26] It is, however, important to note that lymphocyte infiltration in skin or enlargement of lymph nodes is also a consequence of a cutaneous infection that triggers an immune response.[27] Hence, it is possible that the immunohistological evidence for immune involvement cited above is mediated by a concurrent infection rather than a direct response to the drug itself. Of interest, it appears that viral infection may itself be a predisposing factor for the development of CDRs.[4]

The most compelling evidence supporting the involvement of the immune system in CDRs is provided through the demonstration of the proliferation of drug-specific T lymphocytes obtained from patients with a history of CDR. Pichler and colleagues have been able to isolate drug-specific CD4+ and CD8+ T-cell patients with a history of CDR to sulfonamides, cephalosporins, and anticonvulsants.[28-30] T-cell clones could not be isolated from patients

with a drug ingestion history but no history of CDR. Subsequently, similar observations have been made for additional drugs by other investigators.[31] Further support has been provided by Roujeau and colleagues, who have demonstrated the presence of CD8+ cytotoxic drug-specific T cells in blister fluid of patients suffering from toxic epidermal necrolysis.[32,33] In addition, Schnyder et al[34] were able to demonstrate T cell-mediated killing of interferon-γ treated keratinocytes isolated from a patient with a history of a CDR during treatment with sulfamethoxazole. Together, these studies provide substantial evidence for the role of T cell-mediated immunity in DTH skin reactions to drugs.

Critical Steps in the Provocation of Immune Reactions to Xenobiotics in the Skin

Substantial advances in our understanding of immune responses in the skin have been derived from the study of contact sensitizing agents.[35] These studies reveal a complex sequential series of events that involve the engagement of multiple cell types and modulation by a variety of endogenous chemical mediators (Figure 1). Of importance, most small molecules are not believed to be direct immunogens or antigens but instead must first bind (generally covalently) to cellular or extracellular proteins prior to recognition by key immune molecules. These haptens are taken up by antigen presenting cells (APCs) in the skin either by pinocytosis or by receptor-mediated endocytosis. Following hapten uptake, APCs upregulate expression of specific surface molecules (eg, major histocompatability complex [MHC]), costimulatory molecules (eg, CD80), and cytokines.[36] The primary professional APCs in skin are Langerhans cells (LC),[37,38] which are resident dendritic cells (DC) that serve a sentinel function. In response to antigenic challenge, LC migrate to the lymph node carrying the hapten and present it to responsive T cells. Epidermal keratinocytes (KC) also play a pivotal role in the initiation and propagation of

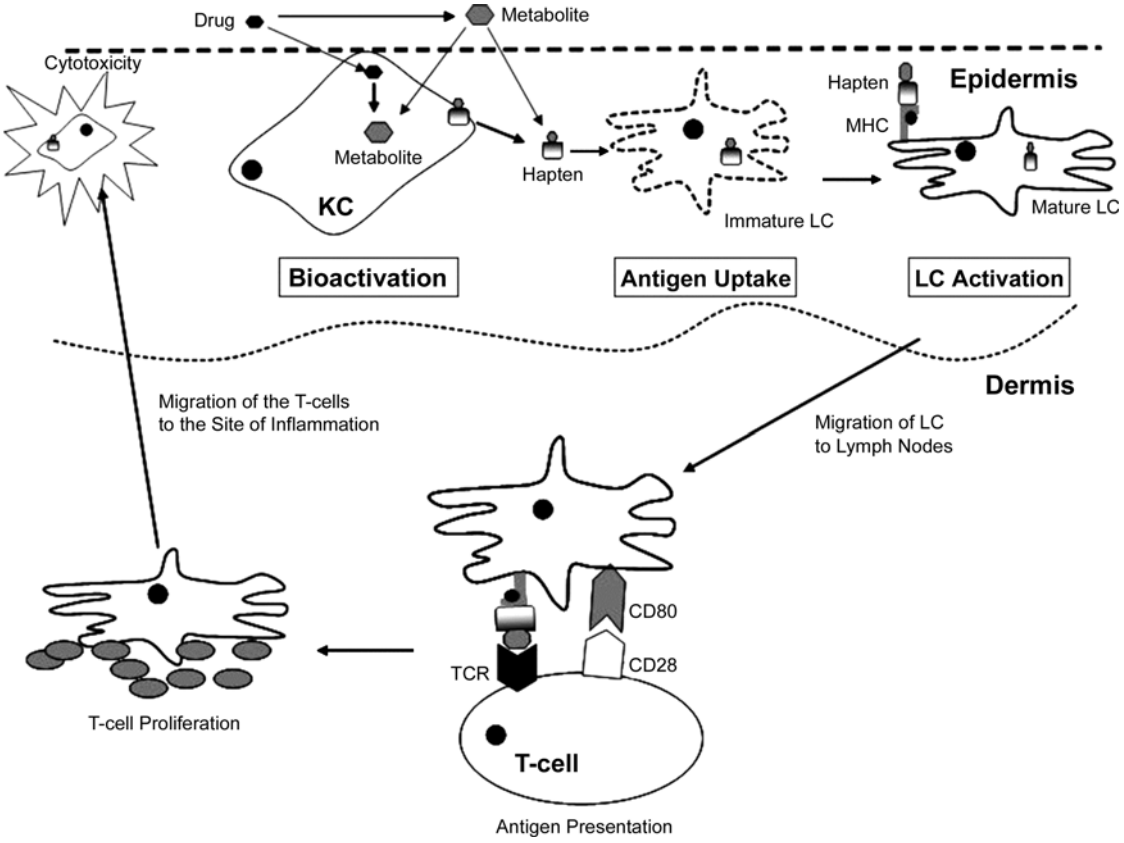

Figure 1. Schematic representation of critical events involved in xenobiotic-induced immune reactions in skin. Drug or metabolite enter the epidermis through the stratum corneum and diffuse into KC. Alternatively, drug or metabolite may enter via the systemic circulation. Drug may undergo bioactivation, followed by intracellular protein haptenation. Metabolite entering directly or released from KC may haptenate extracellular proteins. Antigen uptake by Langerhans cells,[37] together with inflammatory signals, results in activation/maturation of LC. Activation of LC results in the release of signals that direct migration of these cells to the draining lymph node. Upon arrival at the lymph node, LC present antigen to T cells in an MHC-restricted (major histocompatability) fashion. Engagement of the TCR in the presence of costimulatory signals (CD80/CD28) results in clonal expansion of xenobiotic-reactive T cells. These T cells express skin homing receptors, which play an important role in recruitment to the site of allergen presentation. Activation of xenobiotic-reactive T cells in the skin results in cell killing and inflammation.

immune responses in the skin.[39] Hence, an understanding of the mechanism of CDRs necessitates a consideration of critical events that may occur in the skin, which ultimately provoke the observed drug eruption.

Bioactivation

The preponderance of evidence suggests that formation of drug-protein adduct is the first critical step in the elicitation of an immune response following the administration of a small molecule.[40] However, very few drug molecules are chemically reactive in nature, such that they are able to function as immunogens through direct binding to cellular proteins (eg, cephalosporin). Most drugs are chemically inert and must be metabolized (eg, to epoxides, quinones, or nitroso derivatives) or degraded (eg, penicillin) to reactive species prior to haptenation with cellular proteins. Indeed, a survey of drugs associated with DTH reactions in the skin reveals that all have been found to either be metabolized or degraded to reactive species (Table 1).

While the liver is quantitatively the primary site of bioactivation for most drugs, metabolism may also occur in other organs (eg, skin, kidney, or gut). Hence, drugs can distribute to the skin either as the parent compound or as preformed metabolite. Numerous drug metabolizing enzymes have been found to be present in the skin, including cytochromes P-450,[55] N-acetyltransferase,[48] flavin monooxygenases,[56] and cyclooxygenases.[57] We have demonstrated the ability of epidermal keratinocytes[48] and dermal fibroblasts (unpublished data, May 2005) to bioactivate sulfamethoxazole and/or dapsone, giving rise to intracellular haptenated proteins. Evidence is currently lacking for the bioactivation in skin cells of other drugs associated with CDRs.

Dendritic Cell or Langerhans Cell Activation, Maturation, and Migration

Following entry into the skin, reactive drug or metabolite binds to cutaneous proteins and gives rise to drug-protein adducts, which may subsequently be processed and presented by DC/LC as neo-antigens (Figure 1). Activation of immature DC followed by subsequent migration to draining lymph nodes is a critical step for the induction of drug-induced DTH reactions in the skin and other organs.[58] DC collect antigens in peripheral and visceral sites in the body, process these antigens, and transport them to the lymphoid organ in a well-synchronized fashion.[59,60] During this journey to draining lymph nodes, DC undergo a maturation process.[61] For the induction of DTH in skin, LC serve the primary role of APCs[62] and are present both in the dermis and epidermis of the skin.[63] Following interaction with an antigen (eg, contact allergen), LC become activated and migrate from skin to the regional draining lymph node. Various changes have been reported to occur in LC as a result of antigen exposure, including internalization of surface-MHC-II molecules via endocytosis,[64,65] induction of tyrosine phosphorylation,[66] modulation of various cell surface markers,[67,68] and expression of cytokines.[69,70]

Processing and presentation of antigen by LC is a critical step in determining the nature of the immune response. Antigen processing involves the breakdown of long complex proteins into relatively shorter peptides to which the

Table 1. Drugs Known to Cause Cutaneous Drug Reactions That Have Also Been Shown to Form Chemically Reactive Metabolites*

Drug	Metabolite	Enzyme	Reference
Carbamazepine	Arene oxide Quinoneimine	CYP3A4	41,42
Sulfamethoxazole	Hydroxylamine Nitroso N-chloro	CYP2C9, CYP2E1, CYP3A4, myeloperoxidase	43-45
Dapsone	Hydroxylamine Nitroso N-chloro	CYP2C9, CYP2E1, CYP3A4, myeloperoxidase	45-48
Diclofenac	Acyl glucoronide	CYP2C9	49,50
Lidocaine	3-hydroxylidocaine	CYP2D6, CYP3A4	51
Abacavir	Aldehyde	Alcohol dehydrogenase	52
Phenytoin	Arene oxide Quinone	CYP2C9, CYP3A4	53
Procainamide	Hydroxylamine Nitroso	Unknown	54

*Adapted and modified from Park et al.[40]

hapten may be attached.[71-73] This breakdown enables the peptide to bind loosely (noncovalently) to the MHC within the cell. Antigen disposition is the key step that determines which MHC molecule will present the antigens. Extracellular antigens (eg, contact allergens), which involve capture of antigen in the endosome followed by lysosomal degradation, are expected to be presented via class II MHC. Intracellular antigens (eg, drug-protein adducts formed intracellularly) appear to be preferentially presented in a class I MHC-restricted manner.[74] Herouet et al[75] showed a specific increase of MHC-II expression in LC following application of the contact sensitizer dinitrobenzene sulfonic acid in mice. Hence, upregulation of these antigen-presenting molecules upon exposure to allergens appears to play an important role in mediating DTH reactions.

The critical role played by LC in the development of an immune response requires highly synchronized changes in both phenotype and functionality, including cell-cell interactions.[76] These changes have been shown to be regulated largely by cytokines and other pro-inflammatory signals, such nitric oxide (NO) or reactive oxygen species (ROS). Recently, evidence has been presented that indicates that alterations in DC redox balance induced by chemical allergens may play an important role in triggering the maturation of LC.[77] The potential role of such mediators in CDRs is discussed further in a subsequent section of this review.

Expression of Costimulatory Molecules

After interaction with antigen, LC migrate to the lymph node and present the antigen-MHC complex to responsive T cells (Figure 1). T-cell activation requires at least 2 signals. The first signal is delivered in the form of T-cell receptor (TCR) engagement with peptide/MHC expressed on the surface of an APC. The second signal is provided by costimulatory molecules on the APC interacting with their respective receptors on the engaged T cell. The most widely studied costimulatory molecules B7-1 (CD80) and B7-2 (CD86) bind to their respective receptors on T cells (CD28 and CTLA-4 or CD152), which determines the activation state of the T cell.[78,79] In addition to these well-established costimulatory molecules, recent work has identified additional costimulatory molecules (eg, CD40-CD154, OX-40-OX-40L, RANK-RANKL, and PD-1-PD-L-1).[80] Of importance, LC have been found to express various costimulatory molecules, including CD80 and CD86.[81] In addition, KC are also known to constitutively express several costimulatory molecules (eg, CD80), which are upregulated in response to contact-allergen exposure.[82]

Presentation of peptide-MHC complex by DC/LC to T cells can either lead to activation or tolerance, depending upon the expression of costimulatory molecules. Antigen presentation by steady-state DC/LC lacking full expression of costimulatory molecules fail to drive T cells toward productive activation. Important insight into the significance of costimulatory molecule expression during antigen presentation was recently provided by Hochweller and Anderton,[80] who examined the kinetics of costimulatory molecule expression by both DC and T cells in a study of tolerance versus activation. These investigators demonstrated that CD154, OX-40, PD-1, and RANK-L were all expressed on T cells after administration of either soluble antigen (peptide-ovalbumin) or soluble antigen in presence of lipopolysaccharide (LPS). Higher and longer expression of CD154 and OX-40 were observed during the induction of immunity compared with tolerance. However, increases in CD40, RANK, PDL-1, CD80, and CD86 were only observed after LPS in addition to antigen administration. These data indicate that until and unless *sufficient* expression of costimulatory molecules on DC occurs, T cells are not activated to generate an immune response.

T-Cell Propagation

As described above, LC present antigens to T cells in the lymphatic organs in an MHC-restricted fashion. Antigen encounter and recognition activates the transition of a naive T cell to a memory/effector T cell.[83] As illustrated in Figure 1, clonal expansion of drug-specific T cells gives rise to a population of T cells with skin homing receptors that localize to the skin where effector function is needed.

Detection of drug-specific T cells in peripheral blood and inflamed tissues[84,85] from patients with drug hypersensitivity gives evidence of a direct involvement of T cells in DTH reactions associated with drugs. Several investigators have shown that various drugs and or their metabolites (eg, carbamazepine, sulfamethoxazole, lamotrigine, lidocaine, and mepivacaine) can stimulate T cells via interaction with the TCR in an MHC-restricted manner.[31,85-88] The interaction of these drugs with TCR causes cytokine secretion, proliferation, or cytotoxicity in the reactive T-cell clones.

Recently, Gerber and Pichler have provided evidence for T-cell activation arising from noncovalent drug-T cell interactions.[89] This finding has led to a reconsideration of the essential role of haptenation by small molecules and is discussed in depth in a companion article in this series.[90]

The means by which drug-specific T cells are recruited to the site of antigen entry is an area of ongoing investigation. It has been demonstrated that T cells infiltrating the skin express a unique skin homing receptor known as cutaneous lymphocyte-associated antigen (CLA).[91,92] CLA is a carbohydrate epitope present in skin homing T cells, which binds specifically to E-selectin. CLA+ T cells isolated from nickel-sensitive patients have been shown to be activated in response to nickel exposure, whereas CLA- T cells were not activated.[91] Binding of CLA and E-selectin, which is

important for trafficking of T cells through the activated endothelium, also involves the interaction of additional surface markers.[92]

Most drug-induced DTH reactions exhibit complex and overlapping cytokine/chemokine profiles, where various T cells with distinct functions contribute to the clinical manifestations. Immunohistological data suggest that the pathology of drug-induced skin eruptions may depend on the grade of CD4+ and/or CD8+ T-cell activation and function.[18] In maculopapular or eczematous drug eruptions, the immune response appears to be mediated via CD4+ T-cell activation and MHC-II -restricted drug presentation,[18] whereas CD8+ T-cell activation results in more severe skin symptoms (bullous skin disease).[93] These T cells are associated with distinct cytokine secretion profiles (discussed below).

Potential Role of Keratinocytes in Antigen Presentation

KC play an important role in the initiation and propagation of immune responses in the skin.[94] While they do not possess the ability to present antigen under basal conditions, KC may do so in the presence of inflammatory stimuli.[94] The ability to present antigen may play an important role in the targeting of KC for killing by cytotoxic T cells recruited to the skin in CDRs. Presentation of antigens by KC to T cells necessitates the ability of KC to perform numerous APC-like functions, including antigen uptake, intracellular processing, and presentation in the context of MHC molecules, as well as providing reciprocal interaction of cell surface receptors for costimulatory molecules between T cells and KC.

Studies have demonstrated that KC are able to express both types of major histocompatability complexes (MHC-I and MHC-II).[95,96] While MHC-I is expressed constitutively on the surface of KC, MHC-II is only expressed in response to a pro-inflammatory insult (eg, IFN-γ exposure).[95] It has also been demonstrated that contact allergens induce the expression of the important costimulatory molecule, CD80, in KC.[82] Moreover, expression of intercellular adhesion molecule (ICAM-1) is upregulated in the presence of known contact allergens.[97,98] ICAM-1 is known to mediate antigen presentation in functional APC via interaction with the lymphocyte function-associated antigens (LFA-1)[99] and may exhibit similar effects in KC. While further investigation into MHC-restricted antigen presentation by KC is needed, evidence to date suggests that antigen presentation by these cells may play an important role in mediating CDRs.

Potential Mediators of Delayed-type Hypersensitivity Reactions in the Skin

Cytokine/Chemokines

The cutaneous immune response to drugs is regulated in large part by cytokines/chemokines. Cutaneous cells secrete a wide variety of cytokines/chemokines, many of which are induced by allergen exposure and are pro-inflammatory in nature (tumor necrosis factor α [TNF-α], interleukin [IL]-1, IL-2, and IL-6).[100-102] In addition, neutrophil attractant (IL-8), growth promoting (IL-6, Il-7, IL-15, and granulocyte colony stimulating factor [GM-CSF]), and anti-inflammatory cytokines (IL-4, IL-10) are also secreted in the cutaneous environment.[103,104] Drug specific T cells are also believed to mediate inflammatory skin reactions through the release of various chemokines (eg, RANTES, eotaxin, or IL-8).[93] Several of these cytokines (ie, TNF-α, IL-1β, and IL-18) have been found to be important for the mobilization and migration of LC following exposure to contact allergens.[76,105-107] In addition, IFN-γ and TNF-α have been found in blister fluid from patients of toxic epidermal necrolysis.[108] The observation that these cytokines were present in much higher concentrations in blister fluids than infiltrating mononuclear cells suggests that they are secreted by skin cells (eg, KC) rather than cells recruited to the site.[108] The exact role of cytokines in regulating cell killing, however, remains to be determined.

As described previously, pro-inflammatory cytokines (eg, TNF-α) are among the important signals that upregulate the expression of costimulatory molecules on the surface of APC.[109] Pro-inflammatory cytokines also result in the upregulation of MHC-II in KC, which enables these cells to present antigen in an MHC-II-dependent fashion.[110,111] Numerous contact allergens (eg, nickel, dinitrofluorobenzene) have been reported to induce cytokines (eg, TNF-α, IL-1) in cultured KC.[112,113] Pro-inflammatory cytokines are also induced in epidermal KC and dermal infiltrates from mice following exposure to the allergen trinitrochlorobenzene.[114] Cytokines secreted by KC in vitro are used by some investigators to evaluate the sensitizing potential of small molecular weight compounds.[115]

Evidence to date indicates that the functional characteristics of drug-specific T cells is determined by distinct cytokine profiles.[116] Two major phenotypes of cytokine secreting T cells have been identified: type-1, which secrete IFN-γ, and type-2, which secrete IL-4, IL-5, IL-10, and IL-13.[117] Generally, type-1 T cells are involved in pro-inflammatory effector activities, while type-2 induce allergic responses.[118] ACD is predominantly associated with type-1 T cells with less but significant involvement of type-2 cells.[119] The balance between these 2 types of T cells depends on various factors, including the concentration of the antigen, type of antigen, and the cytokines involved in the early interaction of the T cell and the DC.[120-122]

Potent contact allergens are found to activate IFN-γ secreting type-1 effector T cells, and it appears that IFN-γ plays a major role in propagating the cutaneous inflammatory response.[119] Moreover, neutralization of IFN-γ (using monoclonal antibody) at the time of the challenge with the allergen

(eg, picryl chloride) strongly reduces inflammation.[123] In patients with drug-induced DTH, drug-activated T cells secreted high amounts of type-2 cytokines with normal or low levels of type-1 cytokines.[28]

Nitric Oxide

Nitric oxide (NO) is a small regulatory molecule known to play diverse roles in a wide variety of biological phenomena that can be protective, regulatory, or toxic to cells depending on its intracellular concentration.[124] NO has been implicated in the pathogenesis of various immune-mediated skin diseases. NO is known to exert its effect at multiple cellular and molecular levels that include direct effects on different immune cell types (eg, DC, T cells, neutrophils, or mast cells) either at the site of inflammation or in the lymph node.[125] NO has also been reported to regulate expression of different cytokines and cell surface markers that are essential for the cell-mediated immune response.[126] While the role of NO in cutaneous physiology has been recently reviewed,[127] there has been little consideration of its potential role in mediating CDRs.

NO is synthesized by 3 different enzymes commonly known as inducible NO-synthase (iNOS),[128] endothelial NOS (eNOS), or neuronal NOS. Among these 3 enzymes, iNOS is known to be responsible for the high output NO production in different cell types following inflammatory insults.[129] Of importance, studies have demonstrated that iNOS is expressed in various types of skin cells, including KC,[130] LC,[131] melanocytes,[132] and dermal fibroblasts.[129] KC have also been reported to contain constitutive NOS, which can be activated upon exposure to various stimuli (eg, ultraviolet exposure).[128,133] Moreover, the presence of eNOS has also been demonstrated in the dermal vasculature of skin.[134]

Skin biopsies of patients with actively progressing Stevens Johnson Syndrome (SJS) and toxic epidermal necrolysis (TEN) has shown an upregulation of iNOS in KC and infiltrating inflammatory cells as demonstrated by immunohistochemistry and reverse transcription polymerase chain reaction.[135] It has been suggested that the high amount of NO produced by upregulated iNOS may play a role in the massive epidermal cell death observed in patients with SJS and TEN. In addition to a potential role in epidermal apoptosis and/or necrosis in SJS and TEN, NO is believed to play a regulatory role in elicitation of a cutaneous immune response. For example, immunohistological analysis of skin biopsies from patients with ACD demonstrated expression of iNOS in the upper dermal microvasculature of involved sites.[136] These studies suggest a potential role for iNOS and its product NO in DTH reactions in the skin.

In agreement with these clinical findings, other investigators[137,138] found that the NOS inhibitor L-nitroarginine methyl ester (L-NAME) suppressed allergen-induced contact hypersensitivity (CHS) in mice. Other investigators, however, have not supported these findings.[105] Moreover, the CHS response to dinitrofluorobenzene is enhanced in iNOS-deficient mice compared with healthy controls, suggesting iNOS may normally downregulate the inflammatory response.[125] Recent reports have shown that in vivo encounter with contact allergens upregulates iNOS expression in both KC and LC.[139] However, it is not clear whether this activation of iNOS is a direct consequence of allergen contact or a downstream event. As described previously, exposure to contact allergens causes secretion of cytokines from KC and LC, which are known to upregulate iNOS expression.[140,141] Hence, the observed iNOS induction may be secondary to the release of pro-inflammatory cytokines.

NO, in combination with cytokines secreted from KC/LC following contact allergen exposure, has been suggested to direct various effector cells (eg, T cells, macrophages, neutrophils) to the site of inflammation.[125] Of interest, it has also been demonstrated that intracellular concentration of NO could be a crucial factor in determining its role to direct neutrophils. Wanikiat et al[142] have shown that low NO concentration facilitates chemotaxis of human neutrophils, while high concentration of NO inhibited chemotaxis. Of importance, NO is also known to downregulate expression of several surface molecules (eg, ICAM-1, vascular adhesion molecule [VCAM-1]) that are known to mediate adhesion of leukocytes to vascular endothelial cells and infiltration of leukocytes into the peripheral tissues.[143]

Interesting insight into the potential impact of cutaneous NO production has been provided through the demonstration that NO induces apoptosis of DC and T cells.[144,145] Apoptosis of antigen-presenting DC in the skin or draining lymph nodes would be expected to limit the expansion of antigen-specific T cells,[144] thus downregulating the immune response. NO produced by DC and macrophages in the skin would also potentially induce apoptosis in recruited drug-specific T cells.[146]

Thus, while biopsy samples from patients with acute CDR suggest a potential role for NO/NOS in modulating these reactions, the results of experiments in animal models of CHS are conflicting. While further experimental work is needed to ascertain what role, if any, this potential mediator plays in the mechanism of CDRs, the cumulative evidence to date suggests a role for NO/iNOS in downregulating the cutaneous inflammatory response to antigens.

Reactive Oxygen Species

Reactive oxygen species (ROS) are known to be engaged in several of the critical steps (eg, DC activation/maturation, release of cytokines, T-cell activation) involved in the skin immune response.[120] At sites of inflammation, tissue damage has been linked to the release of various pro-oxidants,[147]

which have the potential to alter DC phenotype/function. These pro-oxidants may either directly affect DC maturation and/or activation[148] or act as a signal to induce expression of different immune regulatory molecules (eg, ICAM-1,[149] MHC-II,[150] cytokines[151]). Moreover, altered antioxidant status in mononuclear blood cells from patients with CDRs[152] suggests a direct involvement of ROS during DTH reactions. Thiobarbituric acid derivatives and carbonyl content (indicators of ROS-mediated cellular oxidative stress) were also found to be elevated in the serum of those patients with CDRs to phenytoin and carbamazepine.[152]

Human immunodeficiency virus (HIV)-infected individuals have been found to be more susceptible to drug-induced cutaneous DTH reactions.[153] A lowered antioxidant (eg, glutathione) status in HIV-infected individuals has been suggested as a predisposing factor in the higher frequency of CDR in this population.[154] Altered antioxidant levels (measured as higher oxidized glutathione) in skin biopsies of ACD patients[155,156] and irreversible inhibition of mammalian thioredoxin reductase by the contact allergen dinitrochlorobenzene[157] also suggest involvement of ROS in CDRs. Exposure to reactive metabolites of sulfamethoxazole and dapsone, drugs known to cause CDRs, have been shown to cause depletion of reduced glutathione,[48] as well as higher ROS generation in epidermal KC[158] and dermal fibroblasts (unpublished data, May 2005). Taken together, these data indicate that reduced antioxidant status and/or higher ROS generation in skin cells may predispose individuals to CDRs.

Among various ROS, superoxide has been shown to induce early maturation of (monocyte-derived) DC via upregulation of cell surface markers (CD80, CD83, and CD86) and to downregulate endocytic activity.[159] Superoxide-induced DC maturation is inhibited in the presence of the antioxidant N-acetylcysteine.[99,159] In contrast, H_2O_2 failed to cause DC maturation and CD86 upregulation.[151,159] However, H_2O_2 has been found to upregulate cytokines (eg, TNF-α, IL-8) by human DC, suggesting that these cells could convey the immune response through enhanced cytokine production as a consequence of oxidative stress.[151]

ROS have also been shown to increase the expression of numerous adhesion molecules (eg, E-selectin, ICAM-1, and VCAM-1).[160] Of importance, N-acetylcysteine reduces ROS-induced ICAM-1 expression in human KC.[161] Expression of E-selectin and VCAM-1 by contact allergens is also prevented in the presence of NAC.[162] These results suggest that ROS are able to regulate the recruitment of T cells to the site of inflammation via alterations in the expression of adhesion molecules.

ROS is also known to influence the DC-T cell cross-talk, one of the most important criteria for antigen presentation. Interaction between DC and T cells is a bidirectional phenomenon, in which DC deliver activation signals to T cells and also receive signals back from the responding T cells, thereby undergoing terminal maturation. Interference in the cellular redox regulation pathways by ROS has been found to influence this bidirectional communication between DC and T cells.[163] In particular, both DC and T cells exhibited an elevated level of ROS following antigen-specific interactions, which was blocked in the presence of an antioxidant (ebselen). Moreover, ebselen also inhibited the DC-induced proliferation and cytokine production by T cells. These results imply that endogenously produced ROS in DC and T cells act as second messengers, which play an important role in altering their cellular functions during antigen presentation.

ROS has been demonstrated to induce cell death in T cells, which can be prevented by the application of antioxidants.[164] Higher levels of ROS are known to cause necrosis, whereas lower ROS levels induce apoptosis.[165] Apoptosis of T lymphocytes triggered by ROS has been shown to be mediated via different cellular processes, including lysosomal membrane destabilization,[166] Fas-ligand activation,[167] and downregulation of Bcl-2.[168]

Although numerous studies have suggested a direct involvement of ROS in the elicitation of immune responses in various DTH reactions, studies have failed to show significant inhibition of ACD by topical or systemic application of antioxidants.[169,170] This discrepancy can be explained by the fact that different ROS may simultaneously trigger different cellular processes in various skin cell types. For example, elevated ROS may cause MHC-II upregulation in DC,[150] but at the same time it may induce apoptosis of T cells[166] and thus provide antagonizing effects. Future research should be directed at elucidating the cell-specific mechanism of ROS generation and function during drug-induced immune reactions in skin.

CONCLUSION

Studies examining the immune response to compounds applied epicutaneously have provided important insight into the mechanism of immune responses to xenobiotics in the skin. These studies have revealed several critical steps that must take place prior to the development of a measurable inflammatory response (ie, a rash). But what do these studies tell us about the mechanism by which drugs administered systemically provoke reactions in the skin? It is clear that the pattern of rash observed in response to systemically administered drugs is usually quite different from that provoked by contact sensitizing agents. However, numerous lines of evidence indicate that the immunological responses display significant similarity. As described in this review, xenobiotic-specific reactive T cells have been isolated from patients with ACD and various forms of CDRs. In addition,

upregulation of important surface molecules known to mediate lymphocyte trafficking and activation have been observed in the skin after epicutaneous administration of contact sensitizers and in biopsies of lesions from patients with CDRs. The congruence of these immunological responses suggests important similarities in the mechanism of ACD and CDRs, providing a rationale for the use of ACD as an important experimental paradigm for mechanistic insight that may then be tested within the experimental and ethical limitations surrounding studies in patients with CDRs. It is anticipated that elucidation of the key mediators of these reactions will provide therapeutic targets for the prevention and management of CDRs. Moreover, the available mechanistic insight should foster the development of preclinical screening approaches to identify those compounds with a high likelihood of provoking such reactions.

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